Soft X-ray Microscopy Development at XM-1

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INTRODUCTION

The high resolution soft x-ray microscope XM-1 at beamline 6.1 has been used with applications in biology, environmental sciences, and materials sciences. Instrumental improvements include the development of a cryo-stage, stereo imaging, and a new condenser zone plate lens, which decreased the exposure times at XM-1 by a factor of 15 and also allowed improved spectral resolution [1].

Biologists utilize both natural (absorption) contrast and specific labeling protocols to study parasites, protein distributions in cells, nuclear structure, algae and bacteria. In collaboration with earth and environmental scientists we study macromolecular structures in humic substances, interactions of microorganisms, and related chemical reactions. Chemical reactions related to cement and concrete, which are important to our infrastructure, are studied in collaboration with civil engineers.

LARGE IMAGE TILES

XM-1 allows us to take thousands of high resolution (43 nm) images a day. The size of each of these images is about 10 µm, larger image fields are now regularly tiled together to provide images of large size. This tiling process utilizes the built in precision and digital control of the XM-1 sample-stages and uses software algorithms originally developed by B. Loo [2]. Figure 1 shows a tiled image mosaic of a dividing epithelial cell in late anaphase.

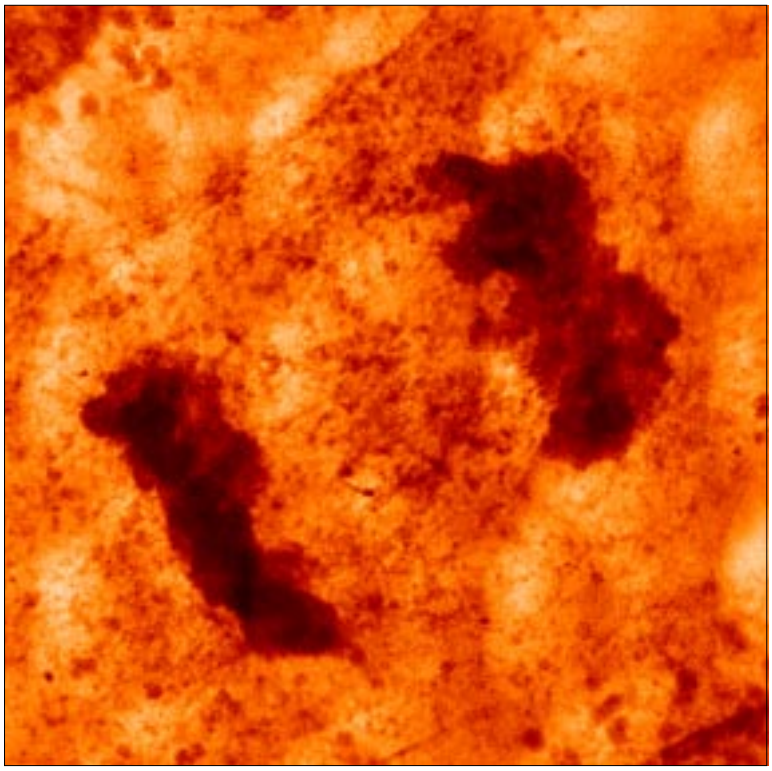

Figure 1. Tiled image of epithelial cells in late anaphase. This image was tiled together from 64 individual images. (courtesy of Sophie Lelièvre, D. Hamamoto, C. Larabell)

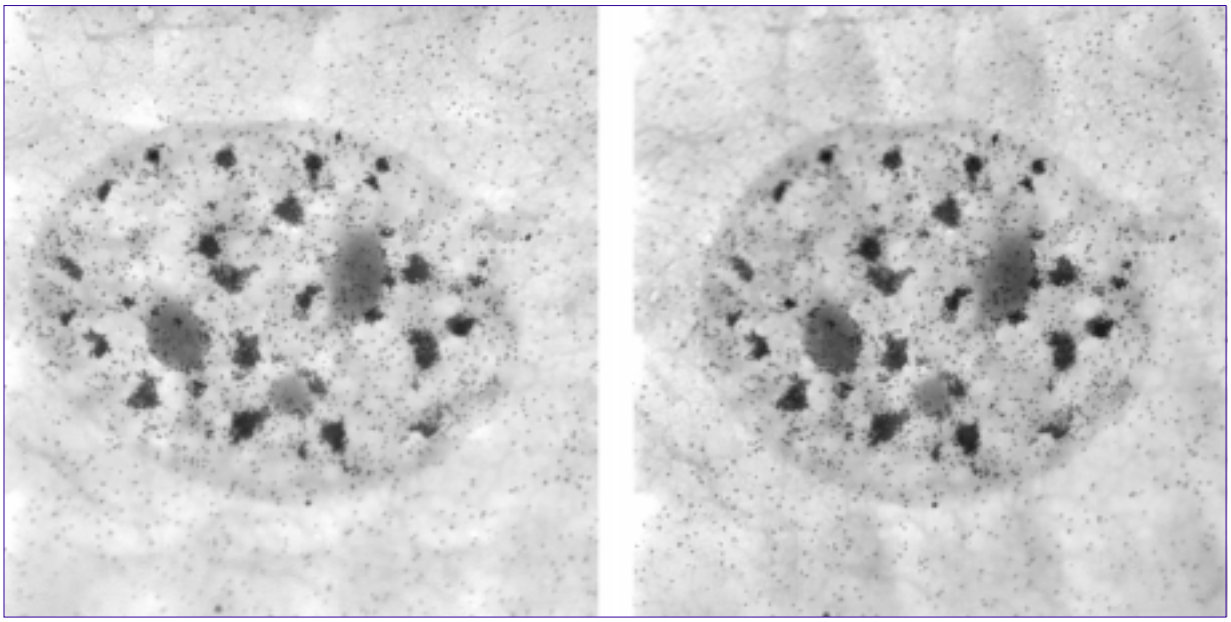

Figure 2: Stereo image of the cell nucleus in an epithelial cell labeled for splicing factor. The sample was fixed by dehydration, which unfortunately does not preserve cell structures. The labeled regions however are clearly visible in 3D, demonstrating the ability to obtain 3D information at high spatial resolution with soft x-rays. Cryo-fixation and observation with a to be developed cryogenic tilt stage would provide the 3D information of the label and the cell structures (courtesy of Sophie Lelièvre, D. Hamamoto, C. Larabell).

3D INFORMATION

Individual x-ray micrographs provide a two-dimensional representation from a three-dimensional sample like a cell. To obtain 3D information, multiple-view images have to be recorded. Two-view images provide stereo sets, and large numbers of views can be used to obtain a full tomographic data set. With a sample tilt stage, XM-1 can be used to take these multiple-view images sequentially. For this to work the sample must not change between the exposures, which cannot be guaranteed with hydrated samples. To demonstrate the 3D capabilities we obtained stereo images of dehydrated cell nuclei that have been labeled with silver-enhanced gold antibodies for RNA splicing factor (Figure 2). To obtain 3D information without dehydration cryo-fixation and observation in a still to be built cryo-tilt stage is needed.

CRYO-STAGE

Cryogenic fixation essentially eliminates structural damage due to the radiation dose needed for multiple high resolution imaging[3]. We have developed a cryo-stage that uses liquid nitrogen cooled helium jets to maintain the sample at temperatures where vitrified ice is stable (below about 130K). Figure 3 shows a green alga (*Chlamydomonas*) images at a frozen state using

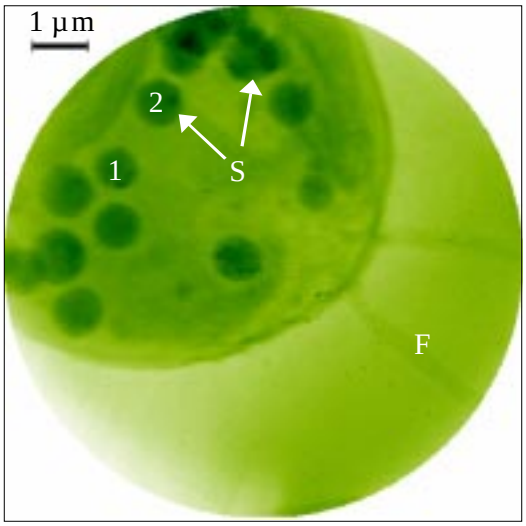

Figure 3: Soft x-ray image of frozen hydrated Chlamydomonas cell. The images show the x-ray absorbing spheres (S), flagella (F) (courtesy of A. Stead, T. Ford, J. Judge).

the cryo-stage in XM-1. Plans exist to develop a cryo-tilt stage that allows to obtain multiple view images in a frozen state as well.

REFERENCES

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[3]G. Schneider, "Cryo X-ray microscopy with high spatial resolution in amplitude and phase contrast," Ultramicroscopy 75 (2), 85-104 (1998).

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